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EXAMINER	
SHEN, WU CHENG WINSTON	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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Office Action Summary	Application No.	Applicant(s)	
	10/585,886	OBERDOERFFER ET AL.	
	Examiner	Art Unit	
	WU-CHENG Winston SHEN	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *05 October 2009*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-36* is/are pending in the application.

4a) Of the above claim(s) *13-17 and 24-36* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-12 and 18-23* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *07/12/2006*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application.
6) ☐ Other: _____.

DETAILED ACTION

This application 10/585,886 is a 371 of PCT/US05/03104 filed on 01/21/2005 which claims benefit of 60/538,871 filed on 01/22/2004.

Election/Restriction

Applicant's election with traverse of Group I, claims 1-12 and 18-23, drawn to a nucleic acid molecule comprising: an RNA polymerase III promoter sequence; a short RNA encoding sequence comprising a transcription initiation site; a *lox*P-flanked STOP cassette comprising an RNA polymerase III-specific termination sequence, a first *lox*P sequence, and a second *lox*P sequence, wherein (i) each of the two *lox*P sequences comprises a spacer region, (ii) the termination sequence is disposed between the first and second *lox*P sequences, and (iii) the termination sequence is disposed between the promoter sequence and the transcription initiation site of the short RNA encoding sequence in the nucleic acid molecule, and an eukaryotic cell comprising the said nucleic acid molecule, in the reply filed on 10/05/2009 is acknowledged. With regard to further restriction to a specific SEQ ID No, Applicant elected SEQ ID No: 3. The traversal is on the ground(s) that SEQ ID NOs: 1-7 are related to one another and should not be subject to restriction. The sequences of SEQ ID NOs: 2-7 can all be found within the sequence of SEQ ID NO: 1, i.e., SEQ ID NOs: 2-7 are all portions of SEQ ID NO: 1 (623-nucleotide long polynucleotide). Accordingly, applicants argues that the restriction requirement with respect to the sequences be reconsidered and withdrawn. This is not found persuasive because the elected SEQ ID NO: 3 (5'-TATAA-3') is only five-nucleotide long, and SEQ ID NO: 3 is clearly not an obvious variant of SEQ ID Nos: 1, 2, 4, 5, 6, and 7. It is further noted that SEQ ID No: 2-7 are

various portions of SEQ ID NO: 1 and SEQ ID NO: 2-7 are patentably distinct sequences. The only certain relationship for SEQ ID NOs: 1-7 recited in the limitation of claim 12 pertaining to "a nucleic acid comprises SEQ ID NO: X" is that "a nucleic acid comprises SEQ ID NO: 3", the elected SEQ ID No, is a genus that encompasses the limitation "a nucleic acid comprises SEQ ID NO: 1".

Claims 1-36 are pending. Claims 13-17 and 24-36 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected invention, there being no allowable generic or linking claim. Claims 1-12 and 18-23 are currently under examination.

The requirement is still deemed proper and is therefore made FINAL.

Drawings

1. The replacement drawings dated 11/01/2006 are objected to because these drawings are not acceptable because they are not in compliance with 37 CFR 1.121(d). Replacement drawings should be so-labeled.

Corrected drawing sheets in compliance with 37 CFR 1.121(d) are required in reply to the Office action to avoid abandonment of the application. Any amended replacement drawing sheet should include all of the figures appearing on the immediate prior version of the sheet, even if only one figure is being amended. The figure or figure number of an amended drawing should not be labeled as "amended." If a drawing figure is to be canceled, the appropriate figure must be removed from the replacement sheet, and where necessary, the remaining figures must be renumbered and appropriate changes made to the brief description of the several views of the

drawings for consistency. Additional replacement sheets may be necessary to show the renumbering of the remaining figures. *Each drawing sheet submitted after the filing date of an application must be labeled in the top margin as either "Replacement Sheet" or "New Sheet" pursuant to 37 CFR 1.121(d).* If the changes are not accepted by the examiner, the applicant will be notified and informed of any required corrective action in the next Office action. The objection to the drawings will not be held in abeyance.

Claim Objections

2. Claim 12 is objected to for being drawn to a non-elected invention. Specifically, Applicants have elected "SEQ ID No:3" as the SEQ ID No recited in claim 12 and as such, claim 12 is examined only to the extent that they read on a SEQ ID No:3. Applicants are required to delete the non-elected subject matter from the instant claim.

Claim Rejection - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless -

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

(e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

3. Claims 1, 3, 6-9, 11, 18-20, 22, and 23 are rejected under 35 U.S.C. 102(a) as being anticipated by **Kasim et al.** (Kasim et al., Control of siRNA expression utilizing Cre-loxP recombination system, *Nucleic Acids Res Suppl.*, (3):255-6, 2003; This reference is cited in the IDS filed by Applicant on 07/12/2006).

Claim 1 reads as follows: A nucleic acid molecule comprising: an RNA polymerase III promoter sequence; a short RNA encoding sequence comprising a transcription initiation site; a loxP-flanked STOP cassette comprising an RNA polymerase III-specific termination sequence, a first loxP sequence, and a second loxP sequence, wherein (i) each of the two loxP sequences comprises a spacer region, (ii) the termination sequence is disposed between the first and second loxP sequences, and (iii) the termination sequence is disposed between the promoter sequence and the transcription initiation site of the short RNA encoding sequence in the nucleic acid molecule.

With regard to the limitations recited in claims 1, 3, 6-9, 18-20, 22, and 23, Kasim et al. teaches vector-mediated systems for specific siRNA expression in mammalian cells using pol III promoters allowing high level of transcription activity have been developed, widening the usage of RNA interference (RNAi). Kasim et al. teaches the controlled pol III promoter (U6 promoter)-driven expression of siRNA using the Cre-loxP system, and the "Cre-On" siRNA-expression vector against firefly luciferase activity taught by Kasim et al. could be switched on only in the presence of Cre recombinase, which, in the study, was delivered directly from the medium into the mammalian cells as TAT-NLS-Cre, a fusion protein with TAT peptide (an Arg rich peptide derived from HIV) and nuclear localizing signal (NLS). Kasim et al. teaches that upon the addition of TAT-NLS-Cre, complete and functional siRNAs were generated and reporter activity was suppressed (See abstract, Kasim et al., 2003).

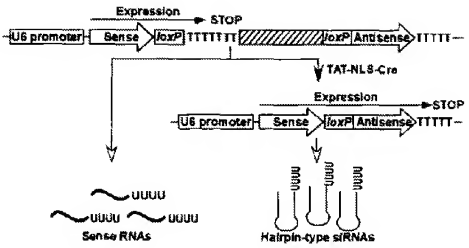


Fig. 1 Schematic representative of the switching-on of Cre-On siRNA expression vector: open arrows show transcription, and close arrow shows recombination. Only in the presence of the Cre recombinase complete, functional siRNAs are formed.

Kasim et al. teaches that to achieve the purpose of establishing a Cre-controllable U6 promoter-based siRNA-expression vector, Kasim et al. designed a Cre-On siRNA expression vector by placing, between sense and antisense regions of siRNA encoding sequence, two loxP sites with an 813 bp insert fragment between them. Kasim et al. teaches that the inserted fragment starts with seven thymines, and as four consecutive thymines is a terminator sequence for the U6 promoter in mammalian cells, and in the absence of Cre recombinase, only sense RNAs, which have no suppressive effect, are transcribed. Kasim et al. teaches that in the presence of Cre recombinase, as recombination between the two *loxP* sites occurs and the inserted fragment, including the termination sequence, is excised, only a 34-bp loxP site separates the sense and antisense sequences. As the result, complete and functional hairpin-type siRNA with a 34-nt loop (loxP sequence, i.e. two 13-nucleotide repeats with 8 nucleotide spacer

in between the repeats) are transcribed (See bridging paragraph, pages 255-256, and Fig. 1, provided above, Kasim et al.).

With regard to the limitation "wherein the short RNA encoding sequence encodes a transcript with fewer than 30 nucleotides recited in claim 11, Kasim et al. teaches the products of this siRNA expression vector are 21-nt hairpin-type siRNAs with a 9-nt loop each. The iGL3BCre-On was designed for the Cre-On system (Fig. 1) with the same sense and antisense sequences as those of iGL3B, while iGL3BloxP was similar to iGL3B, with the exception that the loop consisted of a 34-nt loxP sequence (See left column, page 256, Kasim et al., 2003).

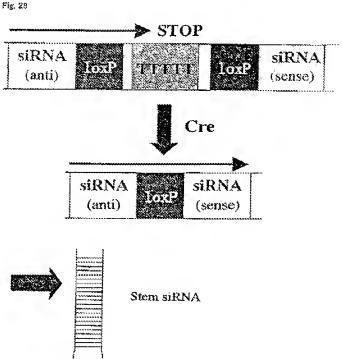
Thus, Kasim et al. clearly anticipates the claims 1, 3, 6-9, 11, 18-20, 22, and 23 of instant invention.

4. Claims 1, 3, 5-12, 18-20, 22, and 23 are rejected under 35 U.S.C. 102(e) and are rejected 35 U.S.C. 102(a) as being anticipated by **Taira et al.** (Taira et al., US 2004/0002077, publication date 01/01/2004, filed on 11/27/2002).

Claim 1 reads as follows: A nucleic acid molecule comprising: an RNA polymerase III promoter sequence; a short RNA encoding sequence comprising a transcription initiation site; a loxP-flanked STOP cassette comprising an RNA polymerase III-specific termination sequence, a first loxP sequence, and a second loxP sequence, wherein (i) each of the two loxP sequences comprises a spacer region, (ii) the termination sequence is disposed between the first and second loxP sequences, and (iii) the termination sequence is disposed between the promoter sequence and the transcription initiation site of the short RNA encoding sequence in the nucleic acid molecule.

With regard to limitation of claims 1, 3, and 6, **Taira et al.** teaches *in vivo* siRNA expression system according to this invention is a system that intracellularly expresses small

interfering (si) RNAs and comprises antisense and sense code DNAs coding for antisense and sense RNAs targeting any region of a target gene mRNA and one or more promoters that function to express the antisense and sense RNAs from the antisense and sense code DNAs, respectively (See abstract, Taira et al, 2004). Taira et al. teaches in Fig. 28 is a diagram representing the stem-loop siRNA expression system containing two loxPs that interpose the linker portion containing the stop sequence (See paragraph [0098], Taira et al, 2004).



With regard to the limitations of claim 5 pertaining to relative positions of the first loxp and the second loxP sequences, Taira et al. teaches (a) the promoter comprises distal sequence element (DSE) and proximal sequence element (PSE) with a space there between, and in the space two loxP sequences, one in the vicinity of DSE and the other in the vicinity of PSE; (b) the promoter comprises DSE and PSE that are located to maintain the promoter activity, a loxP sequence there between, and another loxP sequence either upstream of DSE or downstream of

PSE; and (c) two loxP sequences are located so as to interpose the antisense code DNA or sense code DNA (See paragraphs [0025]-[0027] and claim 14, Taira et al., 2004). Taira et al. teaches (a) the promoter comprises DSE and PSE with a space there between, and in the space two loxP sequences, one in the vicinity of DSE and the other is the vicinity of PSE; (b) the promoter comprises DSE and PSE that are located to maintain the promoter activity, a loxP sequence there between, and another loxP upstream of DSE or downstream of PSE; (c) two loxPs are located so as to interpose the antisense code DNA or sense code DNA; and (d) two loxPs are arranged so as to interpose a linker comprising a stop sequence (e.g. TTTTT) (See paragraphs [0031]-[0034] and claim 17, Taira et al., 2004). Taira et al. teaches that it is also possible to insert an *arbitrary spacer sequence* between the antisense and sense RNA producing units so as to adjust the distance between the two RNAs expressed by the respective units (See paragraph [0114], Taira et al, 2004). Taira et al. teaches that *there is no particular limitation in the length and sequence of the linker DNA*, which may have any length and sequence as long as its sequence is not the termination sequence, and its length and sequence do not hinder the stem portion pairing during the mature RNA production as described above. As an example, DNA coding for the above-mentioned tRNA and such can be used as a linker DNA (See paragraph [0110], Taira et al., 2004). Taira et al. further teaches at nucleotide level the construction of siRNA expression constructs (See Figures 19-25, paragraphs [0083]-[0095], Taira et al., 2004).

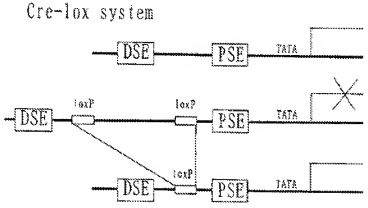
With regard to RNA polymerase III promoter recited in claim 1 and U6 transcription termination sequences recited in claims 7-10, Tiara et al. teaches vector expressing stem-loop siRNA using human U6 promoter (pU6stem) (See paragraph [0251], Taira et al, 2004). Taira et al. teaches that if an inducible promoter is used as the promoter in this invention, siRNA can be

expressed at any desired timing. Such inducible promoters include the tetracycline-inducible U6 promoter (Ohkawa, J. & Taira, K. Control of the functional activity of an antisense RNA by a tetracycline-responsive derivative of the human U6 snRNA promoter. *Hum. Gene Ther.* 11, 577-585 (2000); Fig. 12). In addition, siRNA expression may be tissue-specifically induced using a tissue-specific promoter or a DNA recombination system such as Cre-loxP system (See paragraph [0116], Taira et al, 2004). Taira et al. teaches that in the case of the stem-loop siRNA expression system, it is possible to provide two loxPs in the linker portion so as to interpose the stop sequence (e.g. TTTTT, four consecutive thymines is a terminator sequence for the U6 promoter in mammalian cells). Without CRE protein, transcription from the promoter is terminated at the stop sequence in the linker portion, leading to the suppression of siRNA production. CRE protein induces the recombination between loxPs to displace the stop sequence, leading to transcription of antisense and sense code DNAs to produce the stem-loop siRNA (cf. Fig. 28) (See paragraph [0118], Taira et al, 2004). Taira et al. teaches, as an example, to the blunt end side (EGFP cDNA fragment side) of this product, the 5'-end phosphorylated DNA linker 2 was ligated (FIG. 20-[circle over (4)]). AAAAA/TTTTT sequence signaling the termination of transcription from Pol III promoter is present at the one end of this DNA linker, while the AscI recognition site is present at the other end, and only the AAAAA/TTTTT side is 5'-phosphorylated (See paragraph [0220], Taira et al, 2004).

With regard to the limitation "wherein the short RNA encoding sequence encodes a transcript with fewer than 30 nucleotides recited in claim 11, Taira et al. teaches in Fig. 24 a diagram representing the preparation of EGFP cDNA fragment of approximately 20 to 25 bp long. The final product that is a random EGFP cDNA fragment of approximately 20 to 25 bp

long with the dephosphorylated blunt end serves as the random DMA fragment in Fig. 19 [circle over (1)] (See paragraph [0094], Taira et al., 2004).

With regard to the limitation of claim 12, the nucleic acid molecule comprises SEQ ID No: 3 (5'-TATAA-3'), Taira et al. teaches target site-dependent gene silencing in Example 3, and antisense strand with nucleotide overhang, 5'-GTATAATACACCGCGCTAC-3' (SEQ ID NO: 10) to silence firefly luciferase gene (See paragraphs [0167] and [0168], Taira et al., 2004). Taira et al. also teaches TATA box present in the promoter that expresses siRNA in the Cre-lox system (See Fig. 17, provided below, Taira et al., 2004).



With regard to an eukaryotic cell and a mammalian cell recited in claims 18-20, 22 and 23, Taira et al., the case of introducing siRNA expression system mainly into mammals has been described above, the system may be used in plants. The RNAi induction by the direct introduction of conventional double-stranded RNA into plant cells is difficult to maintain RNAi effects due to the loss of dsRNA during the cell passage processes (See paragraph [0130], Taira et al., 2004).

Thus, Taira et al. clearly anticipates the claims 1, 3, 5-12, 18-20, 22, and 23 of instant invention.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

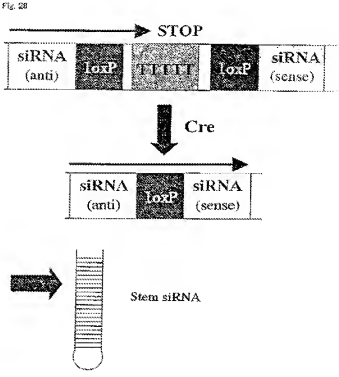
> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

5. Claims 1, 2, and 4 are rejected under 35 U.S.C. 103(a) as being unpatentable over **Taira et al.** (Taira et al., US 2004/0002077, publication date 01/01/2004, filed on 11/27/2002) in view of **Saito et al.** (European Patent Office, EP 1 035 208 A1, publication date 09/13/2000, filed on 11/12/1998).

Claim 1 reads as follows: A nucleic acid molecule comprising: an RNA polymerase III promoter sequence; a short RNA encoding sequence comprising a transcription initiation site; a loxP-flanked STOP cassette comprising an RNA polymerase III-specific termination sequence, a first loxP sequence, and a second loxP sequence, wherein (i) each of the two loxP sequences comprises a spacer region, (ii) the termination sequence is disposed between the first and second loxP sequences, and (iii) the termination sequence is disposed between the promoter sequence and the transcription initiation site of the short RNA encoding sequence in the nucleic acid molecule.

With regard to limitation of claim 1, **Taira et al.** teaches *in vivo* siRNA expression system according to this invention is a system that intracellularly expresses small interfering (si) RNAs and comprises antisense and sense code DNAs coding for antisense and sense RNAs targeting any region of a target gene mRNA and one or more promoters that function to express the antisense and sense RNAs from the antisense and sense code DNAs, respectively (See

abstract, Taira et al, 2004). Taira et al. teaches in Fig. 28 is a diagram representing the stem-loop siRNA expression system containing two loxPs that interpose the linker portion containing the stop sequence (See paragraph [0098], Taira et al, 2004). With regard to RNA polymerase III promoter recited in claim 1, Tiara et al. teaches vector expressing stem-loop siRNA using human U6 promoter (pU6stem) (See paragraph [0251], Taira et al, 2004).



Taira et al. does not explicitly teach a mutant loxP sequence with mutation(s) in spacer region of loxP recited in claims 2 and 4 of instant application.

Saito et al. teaches variant loxP sequences and their application. Saito et al. teaches highly efficient gene integration or gene replacement in the higher eucaryote including animal cells can be performed by using mutant loxP site having the following properties (a)-(c) in the present invention.

(a) a nucleotide sequence wherein, in a wild-type loxP site of the following formula derived from *E. coli* P1 phage, at least one of the bases consisting of second (T), third (G), fourth (T) and fifth (A) bases, and at least one of the bases consisting of sixth (T) and seventh (G) bases within the 8 bases in the central part of the sequence (spacer region) are substituted by different base, and regions except for the spacer region are optionally substituted by any base;

12345678

5'-ATAACTTCGTATA ATGTATGC TATACGAAGTTATL-3'

Spacer Region

(b) a specific recombination between said mutant spacer and the wild-type loxP site can not occur even in the presence of recombinase Cre; and

(c) a specific recombination between the mutant spacer sites having identical nucleotide sequences can occur in the presence of recombinase Cre (See title and abstract, Saito et al., 2000).

Therefore, it would have been *prima facie* obvious to one having ordinary skill in the art at the time of the invention to combine the teachings of Taira et al. regarding the stem-loop siRNA expression system containing two wild-type loxPs that interpose the linker portion containing the stop sequence, with the teachings of Saito et al. regarding a mutant loxP sequence with mutation(s) in spacer region of loxP results in highly efficient gene integration or gene replacement in the higher eucaryote including animal cells, to arrive at claims 1, 2, and 4 of instant application.

One having ordinary skill in the art would have been motivated to combine the teachings of Taira et al. with the teachings of Saito et al. because Saito et al. teaches a mutant loxP sequence with mutation(s) in spacer region of loxP results in highly efficient gene integration or gene replacement in the higher eucaryote including animal cells, which can be used to improve the stability and fidelity of the siRNA expression construct taught by Taira et al.

There would have been a reasonable expectation of success given (i) the successful demonstration of the stem-loop siRNA expression system containing two wild-type loxPs that interpose the linker portion containing the stop sequence, by the teachings of Taria et al., and (ii) demonstration of a mutant loxP sequence with mutation(s) in spacer region of loxP results in highly efficient gene integration or gene replacement in the higher eucaryote including animal cells, by the teachings of Saito et al.

Thus, the claimed invention as a whole was clearly *prima facie* obvious.

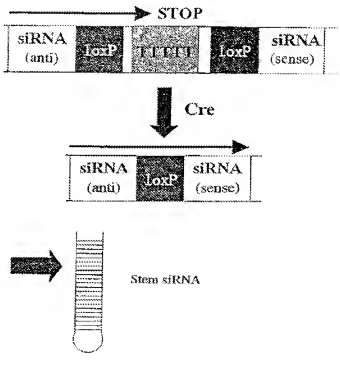
6. Claims 1, 18, 19 and 21 are rejected under 35 U.S.C. 103(a) as being unpatentable over **Taira et al.** (Taira et al., US 2004/0002077, publication date 01/01/2004, filed on 11/27/2002) in view of **Yang et al.** (Yang et al., Specific double-stranded RNA interference in undifferentiated mouse embryonic stem cells, *Mol Cell Biol.* 21(22):7807-16, 2001).

Claim 1 reads as follows: A nucleic acid molecule comprising: an RNA polymerase III promoter sequence; a short RNA encoding sequence comprising a transcription initiation site; a loxP-flanked STOP cassette comprising an RNA polymerase III-specific termination sequence, a first loxP sequence, and a second loxP sequence, wherein (i) each of the two loxP sequences comprises a spacer region, (ii) the termination sequence is disposed between the first and second loxP sequences, and (iii) the termination sequence is disposed between the promoter sequence

and the transcription initiation site of the short RNA encoding sequence in the nucleic acid molecule.

With regard to limitation of claim 1, **Taira et al.** teaches *in vivo* siRNA expression system according to this invention is a system that intracellularly expresses small interfering (si) RNAs and comprises antisense and sense code DNAs coding for antisense and sense RNAs targeting any region of a target gene mRNA and one or more promoters that function to express the antisense and sense RNAs from the antisense and sense code DNAs, respectively (See abstract, Taira et al, 2004). Taira et al. teaches in Fig. 28 is a diagram representing the stem-loop siRNA expression system containing two loxPs that interpose the linker portion containing the stop sequence (See paragraph [0098], Taira et al, 2004).

Fig. 28



With regard to RNA polymerase III promoter recited in claim 1, Tiara et al. teaches vector expressing stem-loop siRNA using human U6 promoter (pU6stem) (See paragraph [0251], Taira et al, 2004).

Taira et al. does not explicitly teach wherein the cell is an embryonic stem cell recited in claim 21 of instant application.

Yang et al. teaches the feasibility of the RNAi strategy in several mammalian cells by using the enhanced green fluorescent protein gene as a target, either by in situ production of dsRNA from transient transfection of a plasmid harboring a 547-bp inverted repeat or by direct transfection of dsRNA made by in vitro transcription. Yang et al. teaches that this long dsRNA was capable of inducing a sequence-specific RNAi for the episomal and chromosomal target gene in undifferentiated embryonic stem (ES) cells, and dsRNA at 8.3 nM decreased the cognate gene expression up to 70%. Yang et al. teaches that the findings offer an opportunity to use dsRNA for inhibition of gene expression in ES cells to study differentiation (See abstract and Figure 5 on page 7813, Yang et al., 2001).

Therefore, it would have been *prima facie* obvious to one having ordinary skill in the art at the time of the invention to combine the teachings of Taira et al. regarding the stem-loop siRNA expression system containing two wild-type loxPs that interpose the linker portion containing the stop sequence, with the teachings of Yang et al. regarding undifferentiated mouse embryonic stem (ES) cells exhibits a sequence specific RNAi at a dsRNA concentration similar to that needed in *Drosophila* S2 cells, and the findings offer an opportunity to use dsRNA for inhibition of gene expression in ES cells to study differentiation, to arrive at claim 21 (which is a dependent claim of claims 1, 18, and 19) of instant application.

One having ordinary skill in the art would have been motivated to combine the teachings of Taira et al. with the teachings of Yang et al. because Yang et al. teaches mouse embryonic stem (ES) cells exhibits a sequence specific RNAi at a dsRNA concentration similar to that needed in *Drosophila* S2 cells, and the findings offer an opportunity to use dsRNA for inhibition of gene expression in ES cells to study differentiation.

There would have been a reasonable expectation of success given (i) the successful demonstration of the stem-loop siRNA expression system containing two wild-type loxPs that interpose the linker portion containing the stop sequence, by the teachings of Taria et al., and (ii) demonstration of specific double-stranded RNA interference in undifferentiated mouse embryonic stem cells, by the teachings of Yang et al.

Thus, the claimed invention as a whole was clearly *prima facie* obvious.

Conclusion

7. No claim is allowed.

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

Any inquiry concerning this communication from the examiner should be directed to Wu-Cheng Winston Shen whose telephone number is (571) 272-3157 and Fax number is 571-273-3157. The examiner can normally be reached on Monday through Friday from 8:00 AM to 4:30

PM. If attempts to reach the examiner by telephone are unsuccessful, the supervisory patent examiner, Peter Paras, Jr. can be reached on (571) 272-4517. The fax number for TC 1600 is (571) 273-8300.

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